ON THE RELATIVE YIELD AND THE DEGREE OF POLARIZATION OF VISCOUS FLUORESCENT SOLUTIONS QUENCHED BY KJ

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It has been experimentally examined how the PERRIN—LEVSHIN and the JABŁOŃSKI equations are fulfilled in case of viscous, quenched solutions. Results obtained show that the degree of polarization as a function of the quotient of absolute temperature and viscosity is not linear in these solutions. It also appears from the result that the relationship between the relative yield and quenching concentration, given in JABŁOŃSKI 's theory of quenching, describes the changes in the relative yield correctly only when the role of diffusion is also taken into account.

1. Introduction

a) The quenching of fluorescence may occur owing to different factors. The different types of quenching (*e. g.* that by concentration, temperature, quenching by absorbing substance etc.) do not occur simultaneously in all substances. However, the quenching by foreign, non absorbing substances is observable at most of fluorescing substances (see *e. g.* [1]). Therefore the process of this type of quenching and its mechanism were studied by many investigators ([2]—[6]). The formulas describing the connections between the relative yield of luminescence and the concentration of quenching substance may be divided into two groups according to the simplifying assumptions used by the different authors in deriving them. In the first group of quenching theories ([2]—[4]) the effect of diffusion on quenching was neglected, consequently relatively simple relationships were given, containing one or two arbitrary parameters between the quantities mentioned above. The second group comprises theories in which also the role of diffusion was taken into account ([5]—[6]). However, formulas derived in the latter way, as a rule, contain more than one arbitrary parameter, thus their comparison with experimental results is more difficult than that of the formulas of the first group.

Therefore it seems justified to examine experimentally the applicability of one of the latest theories belonging to the first group, elaborated by JABŁOŃSKI [4] for the viscous fluorescent solutions quenched by foreign substances. An investigation on this theory seemed all the more reasonable since it contains but one arbitrary factor, the volume of the active sphere.

b) It seemed likewise of interest to examine the validity of the PERRIN—LEVSHIN relationship [7] between the reciprocal value of the degree of polarization $1/p$, the viscosity η_v and the temperature T for viscous quenched solutions. Simple consi-

derations show that in presence of a dynamic quenching substance this relationship apparently looses its validity since $1/p$ is then not a linear function of T/η_v and should give a curve concave from below plotted as a function of T/η_v.

In view of these circumstances the aim of the present work is to investigate experimentally the applicability and validity of both JABŁOŃSKI's and the PERRIN—LEVSHIN relationships for viscous fluorescent solutions quenched by foreign substances.

2. *Experimental*

a) Fluoresceine was used as a luminescent dyestuff in a concentration which produced no concentrational depolarization and in which the Perrin—Levshin relationship was found to be undoubtedly valid for the case of aqueous solutions. In choosing the concentrations of glycerine, the main point of view was to obtain easily measurable degrees of polarization. KJ was chosen as a quenching substance. It is known from the literature that KJ does not absorb in the spectral region under investigation and exerts a dynamic quenching effect. When preparing aqueous glycerine solutions of the dyestuff, NaOH was added to the solution.

The composition of the solutions examined was the following: the concentration of dyestuff, glycerine, and NaOH in all solutions was $1 \cdot 10^{-4}$ mol/l, 60% and 3%, respectively; the concentration of KJ in the solutions being in turn 0, $3 \cdot 10^{-2}$, $1 \cdot 10^{-1}$, $3 \cdot 10^{-1}$, $6 \cdot 10^{-1}$ and 1 mol/l. In this way one unquenched and five quenched solutions were examined.

b) An Optica Milano grating spectrophotometer, Type CF-4 was used to measure the absorption spectra. The tungsten lamp of the apparatus served as an exciting light-source.

Luminescence spectra were obtained with the same spectrophotometer in the following manner: the light of a HBO 200 high-pressure Hg-lamp was transmitted through a SIF 436 mμ metal interference filter, the light beam of the selected spectral range was reflected by a total reflecting prism to a cylindric cuvette of 1 cm thickness containing the fluorescent solution, placed in front of the entrance slit of the spectrophotometer.

Luminescence light of the sample was projected by a lens to the entrance slit of the spectrophotometer. The spectral sensitivity of the photomultiplier of the spectrophotometer was determined previously with a light source of known colour temperature; thus the outer luminescence spectrum could be determined.

The degree of polarization was measured by a photoelectric polarimeter [8] on which the following modifications were carried out in order to increase accuracy of the measurements: 1. a mirror galvanometer of $1{,}2 \cdot 10^{-8}$ A/scale division sensitivity (Type N 91, made in the Soviet Union) was applied as a zero instrument alternatively with a Lindemann electrometer, 2. the temperature of the solutions was determined by a thermistor (of 2 TH 65 Type) in bridge circuit attached to the wall of the cuvette containing the solution.

The determination of the relative yield of the luminescence of solution was carried out according to the method described in [9]. The cuvette containing the solution to be examined being placed in a thermostat, it was possible to determine the dependence of the relative yield on temperature as well.

3. *Results*

Fig. 1 shows the absorption and fluorescence spectra of solutions, the maximum of luminescence spectrum of the unquenched solution being equal to that of the absorption spectrum.

Fig. 1 shows the fact that the shape of the absorption spectra of unquenched and quenched solutions are identical. In Fig. 1 the absorption spectrum of the unquenched solution (○) and the mean of the absorption spectra of the quenched ones (□) are plotted. The luminescent spectra were obtained from the measured outer spectra by correcting them to reabsorption according to [5].

Since we intended to examine the validity of the PERRIN—LEVSHIN relationship for viscous quenched solutions, the degree of polarization was measured with espe-

cial care. The degree of polarization p' was determined at two layer-thicknesses (0,1002 and 0,5024 cm) and at five temperatures (20, 30, 40, 50 and 60° C). The true degrees of polarization p were calculated from the experimental values p' using the following equation:

$$p = \frac{p'}{1 - \varkappa(1 - 0{,}6p')}, \tag{1}$$

where $\varkappa = \varkappa^{(1)}\eta$ is a correction factor for the effect of secondary fluorescence [10]. (The values of $\varkappa^{(1)}$ and η in the factor were determined by spectrophotometric

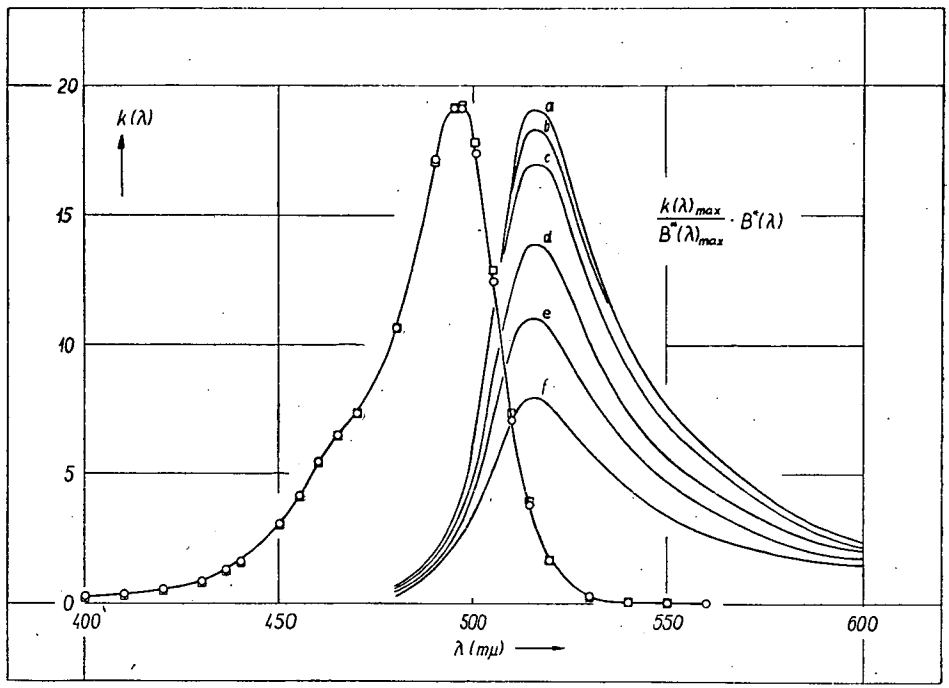

Fig. 1. Absorption and emission spectra [$k(\lambda)$ and $B^*(\lambda)$] of glycerine-aqueous fluoresceine solutions of $1 \cdot 10^{-4}$ mol/l dyestuff concentration containing various amounts of KJ; o—unquenched, □—quenched solutions

measurements.) As to equation (1) the verification of which will be given in a paper to be pulished later, we wish to remark that it is a refined form of Eq. (11/b) in [11].

The true degrees of polarization, determined from experiments repeated several times in the same solution showed very good agreement (within $\pm 1\%$), thus they seem to be applicable for the examination of the validity of the PERRIN—LEVSHIN relationship for viscous quenched solutions (see Table 1.).

Fig. 2 shows the reciprocals of true degrees of polarization as functions of T/η_v. As it may be seen from Fig. 2, function $1/p = f(T/\eta_v)$ is linear in the case of

an unquenched solution, while in cases of quenched solutions it gives concave curves, in agreement with the theory.

Fig. 2 shows also that the slope of curves decreases with the concentration of KJ, indicating the decrease the decay time τ with the concentration of quencher.

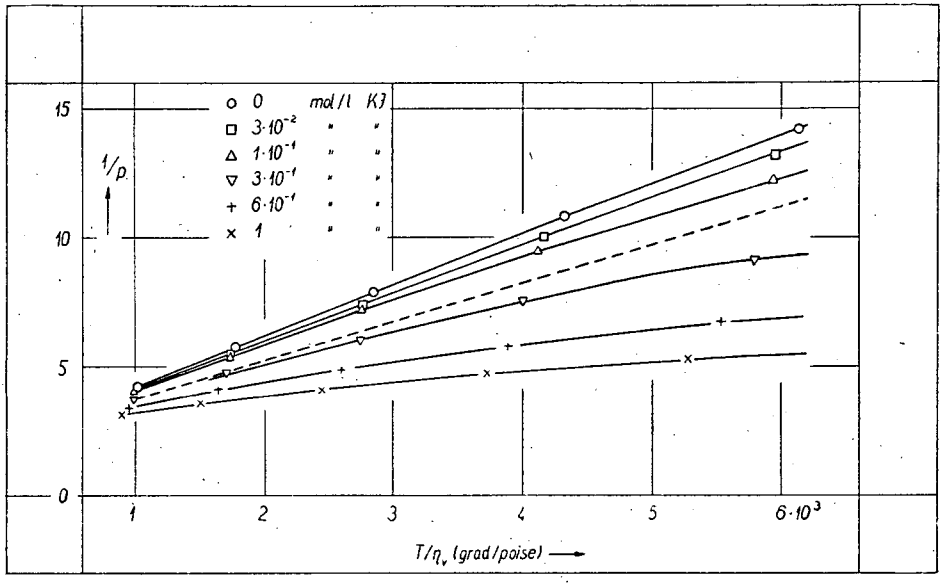

Fig. 2. Reciprocal values of true degrees of polarization $1/p$ versus T/η_v

When quenching luminescence with KJ, it must not be neglected that KJ is a dynamic quencher. Thus the mechanism of such a quenching is to be correctly described only by a relationship which takes into account the influence of viscosity

Table 1

Mean values of true degrees of polarization

c_{KJ} mol/l	20° C	30° C	40° C	50° C	60° C
0	23,86	17,80	12,66	9,26	7,01
$3 \cdot 10^{-2}$	24,71	18,43	13,49	9,99	7,54
$1 \cdot 10^{-1}$	25,00	18,63	13,82	10,53	8,19
$3 \cdot 10^{-1}$	26,84	20,90	16,61	13,25	10,95
$6 \cdot 10^{-1}$	29,39	24,39	20,36	17,27	14,74
1	32,25	27,88	24,07	20,99	18,77

(and consequently the diffusion of quenching molecules) on the change of yield. As a matter of fact, in a dynamical quenching the yield should change in the same direction as viscosity. To give an experimental proof of this statement, relative

yields were measured at different temperature. Table 2 summarizes the relative yields as functions of the quenching concentration and of temperature measured at an exciting light of 495 mμ wavelength (the absorption maximum) and corrected to secondary luminescence, confronted with those calculated from JABŁOŃSKI's equation:

$$\frac{\bar{\eta}}{\eta_0} = \frac{1 - e^{-v}}{v}, \qquad (2)$$

where $\bar{\eta}/\eta_0$ means the relative yield, $v = nv$ is the mean value of the number

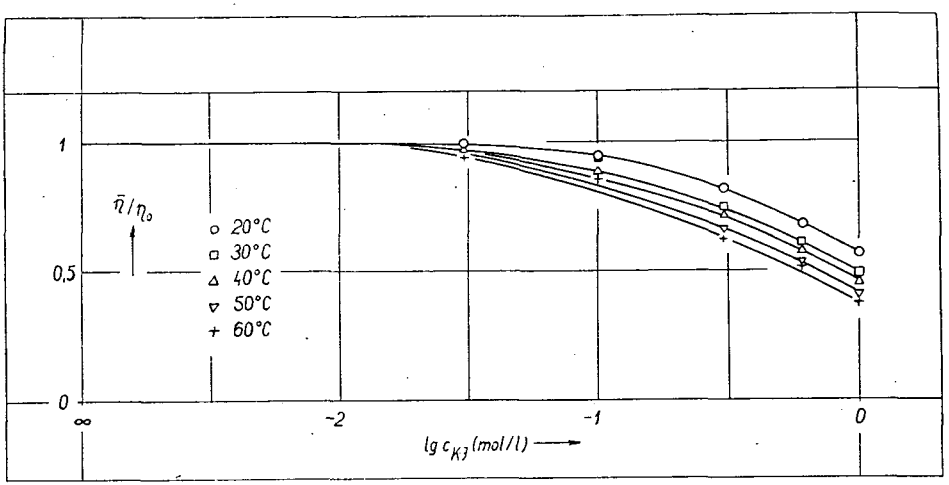

Fig. 3. Values of $\bar{\eta}/\eta_0$ measured at 495 mμ excitation corrected to secondary luminescence versus log c_{KJ}

Table 2

Relative yield measured at 495 mμ excitation corrected to secondary luminescence, calculated with equation (2)

c_{KJ} mol/l	20° C		30° C		40° C		50° C		60° C	
	Meas.	Calc.	Meas.	Calc.	Meas.	Calc.	Meas.	Calc.	Meas.	Calc.
0	1	1	1	1	1	1	1	1	1	1
$3 \cdot 10^{-2}$	0,994	0,981	0,975	0,974	0,971	0,969	0,968	0,963	0,946	0,956
$1 \cdot 10^{-1}$	0,942	0,939	0,933	0,918	0,884	0,901	0,881	0,884	0,859	0,862
$3 \cdot 10^{-1}$	0,818	0,830	0,745	0,780	0,720	0,739	0,660	0,710	0,629	0,656
$6 \cdot 10^{-1}$	0,678	0,697	0,609	0,623	0,580	0,564	0,534	0,514	0,514	0,460
1	0,569	0,561	0,488	0,477	0,459	0,414	0,409	0,364	0,379	0,313

of quenchers in the active sphere of volume v cm^3 at the concentration n cm^{-3} of quenchers. Table 2, and Fig. 3 even more, show very clearly how the relative yields decrease markedly with the increase in temperature (decrease of viscosity),

i. e. the extent of quenching increases together with the increase of diffusion.

„Calculated" values tabulated in Table 2 were obtained in the following manner. JABŁOŃSKI's equation was written in the form:

$$\frac{\bar{\eta}}{\eta_0} = \frac{1 - e^{-ac}}{ac}, \tag{3}$$

where c is the quenching concentration, a an empirical constant adherent to volume v of the effective sphere by $a = 6{,}02 \cdot 10^{20} \cdot v$.

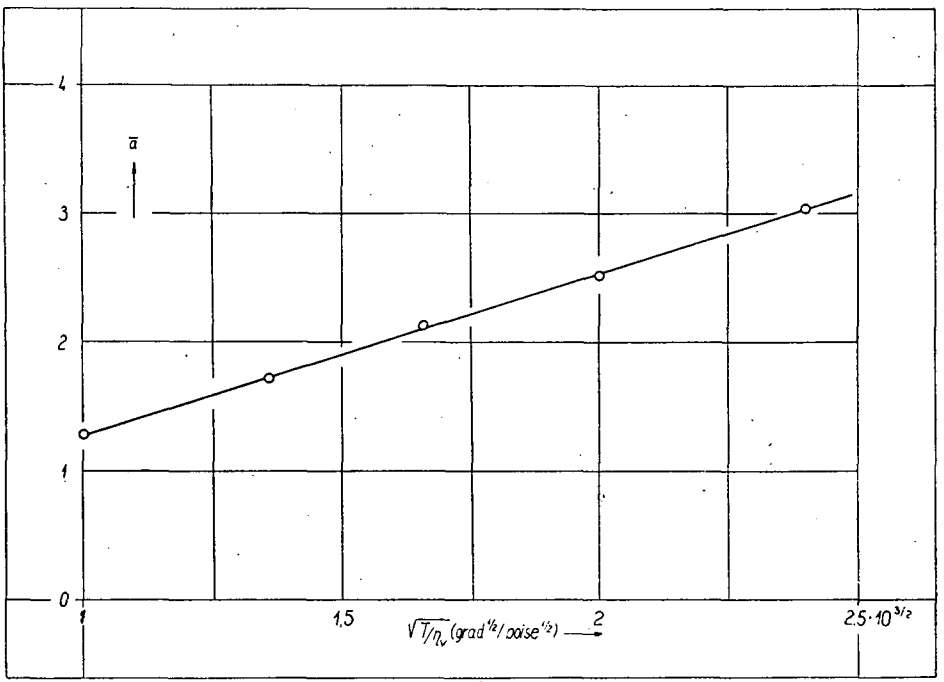

Fig. 4. Values of $\bar{a}$ calculated from $\bar{\eta}/\eta_0$ measured at 495 mμ excitation and corrected to secondary luminescence versus $\sqrt{T/\eta_v}$

Values of a were determined by measuring the relative yields at a given temperature and correcting them for secondary luminescence. After determining the mean values $\bar{a}$ belonging to different quenching concentrations and to the same temperature[1], the relative yields were calculated using Eq. (3).

The determination of values $\bar{a}$ gave a series of $\bar{a}$ and v values increasing with temperature, which indicates that in viscous quenched substances changes of the relative yield are described only approximately by JABŁOŃSKI's equation, since in

[1] Values of a used for the determination of $\bar{a}$ were of $\pm 6\%$ accuracy.

the latter neither temperature nor viscosity are taken into account, as the mutual diffusion of interacting molecules was left out of consideration in its derivation.

The dependence of volume v of the effective sphere on temperature owing to diffusion is supported by the following consideration, which completes JABLOŃSKI's theory by including the diffusion theory. It is known that the Brownian movement of molecules is proportional to the square root of the diffusion constant. Thus, if the abovementioned apparent volume expansion may be ascribed to diffusion, the relationship between v calculated from experimental data by JABLOŃSKI's equation and the square root of the diffusion constant D should be linear with a good ap-

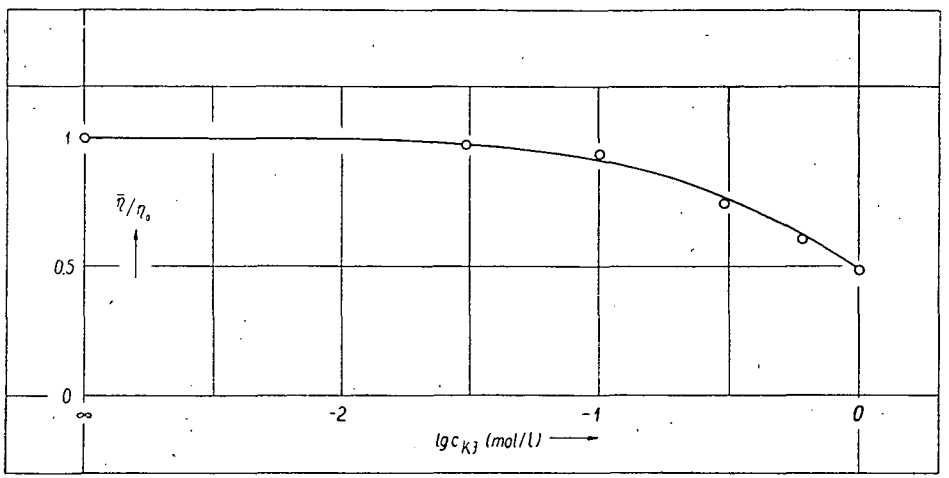

Fig. 5. Calculated (—) and measured (o o o) relative yields versus log c_{KJ}

proximation. According to the theory of dynamic quenching of viscous solutions [5] a and v (proportional to a) may be regarded as consisting of two parts: one, independent of diffusion, being an effective sphere of constant radius around the exciting molecule[2] and a spherical shell of radius $\sqrt{D\tau}$ around the effective sphere which is to be ascribed to diffusion. Therefore

$$a = A + B\sqrt{D}. \tag{4}$$

Substituting for D and reducing constants independent of T and η_v we have

$$a = m\sqrt{\frac{T}{\eta_v}} + A. \tag{5}$$

It is to be noted that both formulas and the above considerations connected with them are valid only in the case of a relatively large viscosity, whenever the volume

[2] The volume of the excited molecule should theoretically be subtracted from the volume of the effective sphere. However, since the latter is independent of η_v and τ, the validity of the following considerations is unaffected by neglecting the subtraction, as it is to be seen by the following equation.

of the mentioned spherical shell can be regarded as proportional to the thickness $\sqrt{D\tau}$ of the spherical shell (which is small in comparison with the radius of the effective sphere). In aqueous solutions with low viscosity the dependence of quenching on viscosity can be interpreted only by special considerations (see pp. 215—216 in [5]).

In Fig. 4 values of $\bar{a}$ calculated from relative yields measured at an exciting light of 495 mμ wavelength are plotted as a function of $\sqrt{T/\eta_v}$. Fig. 4 shows that the expected linearity is well fulfilled in the investigated temperature range, which proves that the dependence of $\bar{a}$ on temperature is really due to viscosity, consequently to changes in the rate of diffusion.

Fig. 5 shows that there exists a good agreement between our experimental data and the theoretical curve if the dependence of values a on temperature and viscosity is taken into consideration, *i. e.* JABŁOŃSKI's equation (2) is modified in the described way. The solid line in the figure is the theoretical curve, calculated with $\bar{a}=1{,}721$ and the circles represent values $\bar{\eta}/\eta_0$ corrected to secondary luminescence measured at the absorption maximum (495 mμ).

4. Conclusions

Experimental results suggest the following conclusions.

a) In case of viscous solutions quenched by foreign substances the Perrin—Levshin relationship apparently looses its validity and in this solutions function $1/p=f(T/\eta_v)$ is not linear.

b) The dependence of the relative yield of viscous, luminescent solutions quenched by KJ on the concentration of the quencher is unequivocally described by JABŁOŃSKI's equation only if the temperature and the viscosity of the solution are unchanged. This is proved by the fact that the volume of the effective sphere changes together with alterations in the temperature and viscosity.

c) If we wish to follow the changes in relative yields of quenched viscous solutions using JABŁOŃSKI's equation, the latter should be modified by taking into account the influence of interacting molecules.

d) The fact that increasing concentration of KJ does not affect the form of either the absorption or luminescence spectra proves on the one hand that KJ is not an absorbing quencher and on the other hand that no observable chemical interaction occurs between the molecules of the solution with increasing concentration of KJ.

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О СТЕПЕНИ ПОЛЯРИЗАЦИИ И ОТНОСИТЕЛЬНОМ ВЫХОДЕ ВЯЗКИХ РАСТВОРОВ ФЛУОРЕСЦЕИНА, ПОТУШЕННЫХ ИОДИДАМ КАЛИЯ

Я. Хевеши

В работе экспериментально исследовалось выполнение соотношения Террина-Левшина и Яблоньского для вязких, потушенных растворов. Получилось, что степень поляризации, как функция частного абсолютной температуры и вязкости при этих растворов имеет нелинейный ход. Также получилось, что соотношение между относительным выходом и концентрацией тушения, в теории потушения, описанная Яблоньским, отрожает правильно изменения относительного выхода только с учётом роли диффузии.